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Primary Recurrent Miscarriages: Anti–β2-Glycoprotein I IgG Antibodies Induce an Acquired Activated Protein C Resistance That Can Be Detected by the Modified Activated Protein C Resistance Test

To the Editor:

Using the study of affinity-purified IgG phospholipid-dependent inhibitors on the time-course of factor Va generation and inactivation, Galli et al demonstrated convincingly that anti–β2-glycoprotein I antibodies induce an acquired resistance to activated protein C (aPC), thus providing a new possible explanation for their thrombogenic potential.

We have studied the effect of various antiphospholipid IgG antibodies on the activated protein C-mediated factor Va proteolysis (aPC-FV) using the modified aPC-resistance test that includes predilution of patient plasma in factor V-depleted plasma, according to Jorquera et al2 (Coatest APC Resistance V; Chromogenix, Mölndal, Sweden; results given as aPC-FV ratios: time obtained in the presence of aPC divided by the time obtained, using the same plasma, in absence of aPC).

All patients were nonthrombotic young women who had experienced primary unexplained recurrent miscarriages. They had been controlled to be negative for the R506Q (factor V Leiden) mutation. Lupus anticoagulant activity (LA) had been tested according to the revised

Table 1. Effect of Various Plasmas Positive for Antiphospholipid-Related Markers on the AP C-Mediated Factor Va Proteolysis (aPC-FV) Using the Modified aPC-Resistance Test

<table>
<thead>
<tr>
<th>aPC-FV Ratio</th>
<th>Patient Plasmas</th>
<th>Mixing Studies 1:1</th>
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</thead>
<tbody>
<tr>
<td>Control plasmas (n = 200)</td>
<td></td>
<td></td>
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<tr>
<td>Whole plasmas</td>
<td>2.54 (2.08-3.36)</td>
<td>2.55 (2.19-2.96)</td>
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<tr>
<td>NP (pooled control plasmas)</td>
<td>2.55</td>
<td>2.55</td>
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</tbody>
</table>

| Isolated LA (n = 10) | | |
| Whole plasmas | 2.64 (2.19-3.24) | 2.59 (2.23-2.85) |
| IgG-depleted plasmas | 2.57 (2.11-3.18) | 2.55 (2.28-2.80) |

| Isolated aCLAb (n = 10) | | |
| Whole plasmas | 2.72 (2.31-3.30) | 2.63 (2.35-2.96) |
| IgG-depleted plasmas | 2.68 (2.35-3.22) | 2.60 (2.38-3.01) |

| Isolated a2GPIAb (n = 10) | | |
| Whole plasmas | 1.52 (1.11-2.02) [1] | 1.75 (1.23-2.19) [3] | 1.93 (1.54-2.52) [5] |

| Isolated aFIIAb (n = 2) | | |
| Whole plasmas | (2.76-3.15) | (2.65-2.96) |
| IgG-depleted plasmas | (2.80-3.04) | (2.71-2.82) |

| Isolated aAnVAb (n = 10) | | |
| Whole plasmas | 2.61 (2.25-3.00) | 2.58 (2.36-2.88) |
| IgG-depleted plasmas | 2.50 (2.29-3.05) | 2.55 (2.34-2.79) |

Results are expressed as aPC-FV ratios (time obtained in the presence of aPC divided by the time obtained in absence of aPC) and are given as median value (range). Statistical analysis (Wilcoxon paired t-test): [1]v [2], [3]v [4], and [5]v [6]: P = .005; [1]v [3], [3]v [5], and [1]v [5]: P = .005. All other comparisons were nonsignificant.

Abbreviations: NP, normal plasma; NP-β2neg, normal plasma after β2-glycoprotein I depletion; LA, positive lupus anticoagulant; aCLAb, positive anticardiolipin IgG antibodies; a2GPIAb, positive anti–β2-glycoprotein I IgG antibodies; aFIIAb, positive antiprothrombin IgG antibodies; aAnVAb, anti-annexin V IgG antibodies.
criteria proposed by the Scientific Standardization Committee Subcommittee for Standardization of Lupus Anticoagulant. Anticardiolipin (aCLAb) and anti–β2-glycoprotein I (aβ2GPIAb) IgG antibodies had been tested using commercially available assays (Quanta Lite [Inova Diagnostics Inc, San Diego, CA] and Varelisa β2GPI [Elias, Freiburg, Germany]). Antiprothrombin (aFlAAb) and anti-annexin V (aAnVAb) IgG antibodies had been assayed using enzyme-linked immunosorbent assay research kits (gift from J. Amiral, Diagnostica Stago, Asnières, France). All normal values were obtained using 200 control plasmas assuming that threshold values corresponded to the 99th percentiles. All patient plasmas were tested crude and after IgG depletion using protein A-sepharose. We systematically performed mixing studies using a 1:1 mixture of patient and normal plasma. Normal plasma has been prepared pooling the 200 control plasmas and was used crude (NP) or β2-glycoprotein I depleted after immunoaffinity chromatography (NP-β2neg).

We identified 42 patients with positive antiphospholipid antibody-related markers: 10 patients with an isolated LA (7 with a KCT-type LA and 3 with a dRVVT-type LA according to Galli et al1), 10 patients with isolated aCLAb (levels ranging from 25 to 68 GPL units, normal values lower than 14.5 GPL units), 10 patients with isolated aβ2GPIAb (levels ranging from 42 to 123 U/mL, normal values lower than 14.5 U/mL), 2 patients with isolated aFlAAb, and 10 patients with isolated aAnVAb (Table 1). We observed that plasmas with isolated positive aβ2GPIAb were the only ones to be associated with an acquired resistance to aPC, which disappeared in cases of IgG depletion. These plasmas generated resistance to aPC during mixing studies and the intensity of the inhibitory activity depended on the β2-glycoprotein I content of the normal plasma. We thereafter supplemented NP and NP-β2neg with IgG purified from plasmas with isolated positive anti–β2-glycoprotein I IgG antibodies (3:1; IgG concentration, 10 mg/mL in Tris-buffered saline). An acquired plasma resistance to aPC was induced only in the presence of plasma β2-glycoprotein I (median values and ranges of aPC-FV ratio: NP, 1.59 [1.19–1.88]; NP-β2neg, 2.22 [1.98–2.45]; P = .005; control values using IgG purified from normal plasma: NP, 2.30 [2.05–2.55], NP-β2neg, 2.28 [2.00–2.49]; nonsignificant).

Thus, the modified aPC-resistance test, together with a mixing study performed in this test, can easily allow us to identify the acquired anticoagulant activity acting as an inhibitor of the aPC-mediated factor Va proteolysis. Concerning women with recurrent miscarriages positive for antiphospholipid antibody-related markers, only aβ2GPIAb is related with the impairment of the anti-factor V activity of aPC. This finding is concordant with data shown by Galli et al1 in their group of patients with mainly thrombotic antecedents. If acquired aPC resistance represents a pathogenic mechanism responsible for the clinical risk in a subgroup of antiphospholipid-positive patients, the modified aPC-resistance test should allow to identify plasmas positive for this peculiar anticoagulant activity.

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Antisense RNA Crossing Mitochondrial Membrane?

To the Editor:

In a recent article in Blood, Shirafuji et al1 report the isolation of a cDNA encoding an antisense RNA for subunit I of mitochondrial cytochrome c oxidase (MARCO). Expression of this cDNA in hematopoietic cell lines caused morphological changes and cell death. Although Shirafuji et al convincingly demonstrate that the antisense RNA induced these changes, we would like to issue with the investigators on the suggested mechanism, namely interference with the corresponding mitochondrial gene transcript (COX I).

Animal mitochondria represent a distinct cellular compartment that has its own mitochondrial DNA, encoding 13 proteins, including 3 subunits of cytochrome c oxidase. The mRNAs of the mitochondrial genes are translated on mitochondrial ribosomes. Whereas the protein components required for mitochondrial gene expression (polymerases, ribosomal proteins) are imported into mitochondria, all the necessary RNA components (transfer-RNAs, ribosomal RNAs) are encoded by the mitochondrial genome and are therefore not taken up from the cytoplasm.2 There is no indication of significant DNA or RNA uptake into mitochondria. Even for the nucleos-encode small RNA components of two mitochondrial ribonucleoproteins (MRP-RNAs1 and RNAs2 P1) the import mechanism is unclear and may involve cotransport with the associated proteins. Nonspecific uptake of polynucleotides is unlikely, because the inner mitochondrial membrane is a nonpolar lipid bilayer with only a very limited permeability towards polar molecules. To support an antisense mechanism, the investigators would have to demonstrate that the MARCO-RNA actually crosses the mitochondrial membrane to interfere with the corresponding mitochondrial COX I gene transcript. Second, the investigators should have shown that other mitochondrial transcripts are not affected, because, otherwise, the reported reduction of the COX I mRNA could be interpreted as an unspecific feature associated with early stages of cell death.

Finally, we would like to speculate on an alternative explanation for the findings of Shirafuji et al.3 Because all the mitochondrial genes encode very hydrophobic membrane proteins, they contain nucleotide sequences that are similar to a variety of other membrane spanning proteins. It is therefore conceivable that MARCO could affect the cytoplasmic translation of an important membrane protein that is located in the cytoplasm rather than in the mitochondria. Decreased expression of this protein may trigger cell death, with an associated early change in mitochondrial gene transcription.
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